

**REMARKS**

By this amendment, Applicants have now amended the claims in such a manner as to overcome all outstanding rejections and objections, and to place this case in condition for allowance. In particular, Claim 7 has now been amended to reflect the precise location of the SdrG protein taking into account the flanking sequences shown in the figures, e.g., Figure 3, and described in the specification, and Applicants have now referred to the precise location of the A domain, e.g., as set forth in Figure 5B and its description, and as reflected in new claims 18 and 19. Applicants have also amended Claim 6 into an independent claim. Applicants submit that the application in its present form overcomes all prior rejections and is now in condition for allowance for the reasons set forth below.

In the Official Action, the Examiner rejected Claims 2-5, 7-10 and 13-17 on the basis of the Guss et al. reference, WO 97/48727. This rejection, insofar as applied to the claims as amended, is respectfully traversed and should be withdrawn for the reasons that follow.

In particular, as Applicants have previously pointed out in this application and its parent cases, while the protein in the Guss reference has some similarity to the SdrG protein of the present claims, indeed there are differences between the two, and these differences have been shown to lead to differences in properties. For example, as shown in Attachments A and B, tests of the SdrG protein showed that antibodies raised thereto were very species-specific to the human fibrinogen and did not bind to rat fibrinogen or other fibrinogens. However, as shown in the attached article by Pei and Flock, antibodies to the FIG/fbe protein of the Guss application was shown to bind to

both human and rat fibrinogen. It is thus clear that there are differences between the antibodies to SdrG and the antibodies as disclosed in the Guss international application, and the cited Guss reference thus does not anticipate or make obvious the present claims. Accordingly, Applicants submit that the rejection on the basis of the Guss reference is respectfully traversed and should be withdrawn.

In the Official Action, the Examiner also rejected Claims 2-10, and 13-17 under 35 U.S.C. §102(b) as being anticipated by US Patent No. 6,380,370 to Doucette-Stamm et al. However, the Examiner recognized that Doucette-Stamm was directed to a disclosure of predicted nucleic acid and amino acid sequences, and thus no actual proteins were expressed, much less any antibodies generated thereto. Instead, the Examiner argued that since the reference generally disclosed generation of antibodies to some of the predicted sequences, the claims directed to antibodies as reflected in the present claims were inherently disclosed in the Doucette-Stamm reference. This rejection, insofar as applied to the claims as amended, is respectfully traversed and should be withdrawn for the reasons that follow.

Contrary to the Examiner's position, the Doucette-Stamm reference does not disclose inherently or otherwise any antibodies, much less the antibodies of the present application. In the first place, as indicated above, at no point in the Doucette-Stamm reference does it refer to expressing any proteins, much less producing any antibodies therefrom, and thus one having in hand the Doucette reference would not be pointed to any particular protein at all, much less one that would be able to be expressed and would be able to be antigenic and thus produce antibodies in the first place. In other words, one with possession of the disclosures of the sequences would need to take the

steps of actually attempting to prepare polypeptides from such sequences, and would not know beforehand if such theoretical sequences would result in actual polypeptides, much less antigenic polypeptides that would generate any antibodies. Moreover, the reference nowhere states or suggests the presence of particular regions within any theoretical sequences such as the A domain, and thus there is no disclosure in any of the cited references that would teach or remotely suggest a specific region within the purported polypeptides. As such, the cited references do not disclose or point to in any way any particular region within a sequence, much less the specific A domain sequence of the amended claims.

Accordingly, the references do not teach that any particular polypeptide or protein can be expressed, much less be antigenic, and certainly do not teach or direct one skilled in the art to any specific region from which to generate a specific antibody. As such, the Examiner's position that the reference disclosed antibodies which inherently would recognize the claimed sequences is without support and can only be asserted with the hindsight of Applicants' invention and discovery of the specific SdrG protein and its A domain of this polypeptide as set forth in the present claims. The Examiner has thus not established a *prima facie* case of anticipation each and every element of the claimed invention, as arranged in the claim, since the present elements of the claims are not disclosed either specifically or inherently by a single prior art reference. See *Minnesota Mining and Mfg. Co. v. Johnson & Johnson Orthopedics, Inc.* 976 F.2d 1559, 1565 (Fed. Cir. 1992).

Instead, the Examiner has failed to establish that the cited reference directly or inherently discloses each and every element of the claimed invention, and instead can

only point to theoretical antibodies that conceivably could be generated from the sequence of the cited reference. To the contrary, "it is the examiner's burden to provide evidentiary support for his factual finding" that a purported prior art antibody would bind to specific targets. *Ex parte Kung*, 17 U.S.P.Q.2d 1545, 1548 (USPTO Bd. App. and Int. 1989) (the Examiner's mere "invitation to consult a basic immunology text hardly suffices" to support a 102 rejection wherein not all of the claimed elements were present).

As reflected above, the cited references only disclose theoretical, computer-generated sequences, and do not actually express any proteins, much less any specific regions within those sequences, and thus do not anticipate the presently claimed invention relating to isolated antibodies capable of binding to the amino acids as set forth in SEQ ID NO: 10, and to the A domain located at amino acids 51-598 of amino acid SEQ ID NO: 10. See also *Ex parte Old, et al.*, 229 USPQ 196, 200 ((PTO Bd. App. And Int. 1985) (uncertainty involved in the generation of antibodies meant that no "expected" results could be said to occur from the knowledge of a specific antigen). The Examiner's rejection on the basis of the Doucette-Stamm reference is thus respectfully traversed and should be withdrawn.

Applicants thus submit that the invention as presently claimed is not anticipated nor made obvious by the cited references, and that the Examiner's rejections on the basis of the prior art are respectfully traversed and should be withdrawn.

In light of the amendments and arguments, set forth in detail above, Applicants submit that the present application overcomes all prior rejections and has been placed in condition for allowance. Such action is respectfully requested.

Respectfully submitted,

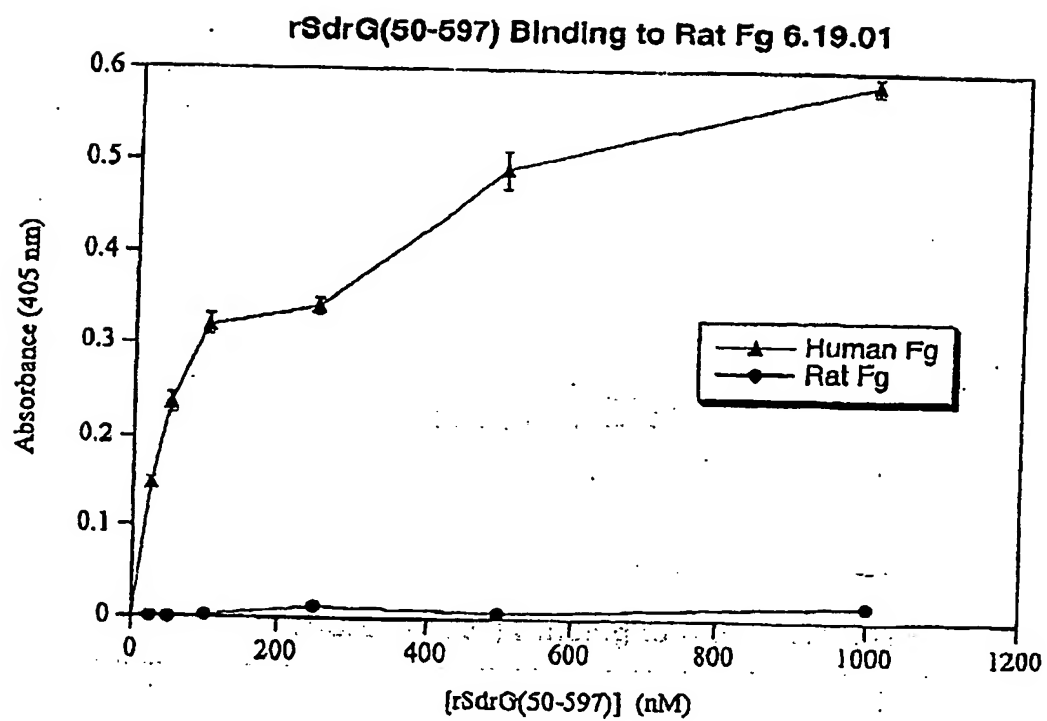
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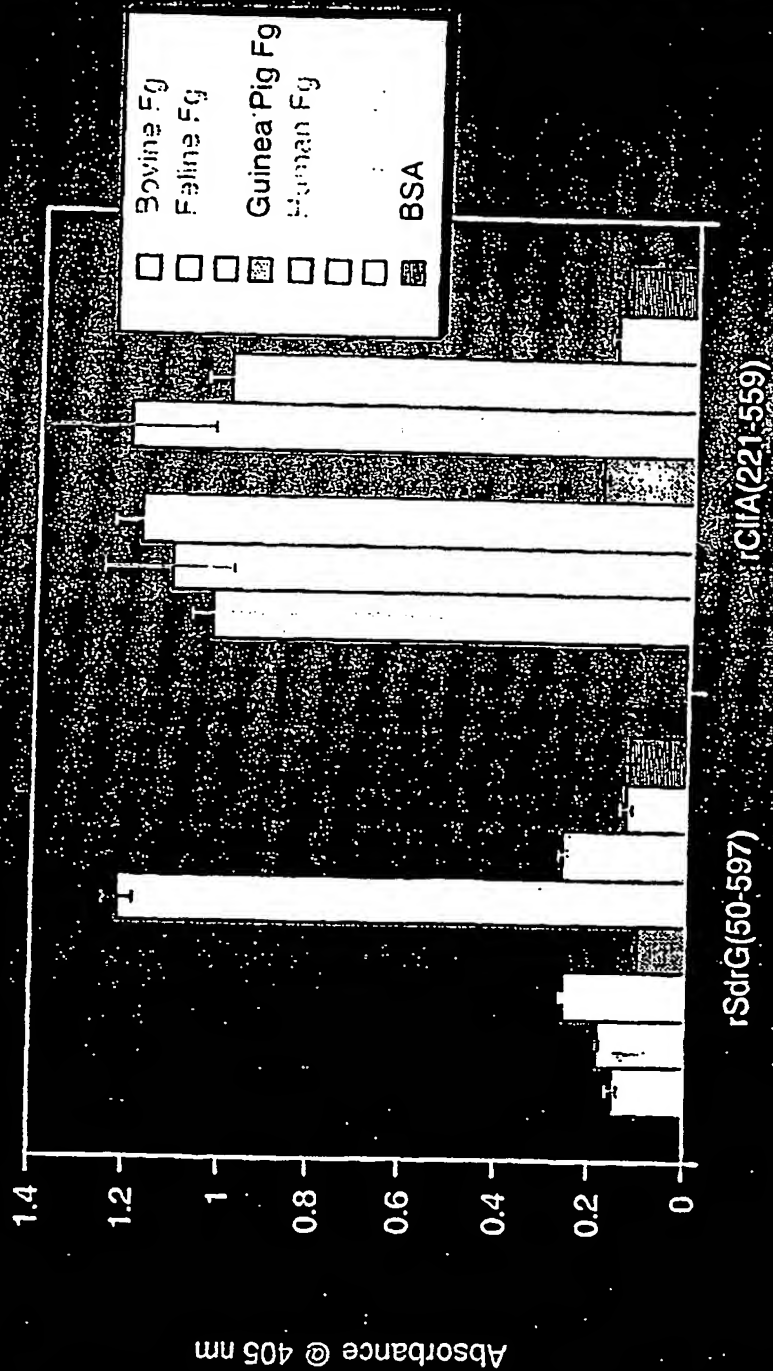
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## ATTACHMENT A



# ATTACHMENT B

## rSdrG(50-597) binding to Fg is species specific



## Functional Study of Antibodies against a Fibrinogen-Binding Protein in *Staphylococcus epidermidis* Adherence to Polyethylene Catheters

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*Staphylococcus epidermidis* is an important pathogen in foreign body-associated infections. In a previous study, we showed that a surface-located fibrinogen-binding protein, termed Fbe, from *S. epidermidis* mediated the bacterial adherence to fibrinogen-coated surfaces in vitro. In the present study, we demonstrate that antibodies against Fbe can block adherence of *S. epidermidis* to fibrinogen-coated catheters, subcutaneously implanted catheters from rats, and peripheral venous catheters from human patients.

*Staphylococcus epidermidis* is the most common coagulase-negative staphylococci colonizing skin and mucous membranes and has become the most commonly reported pathogen in foreign-body infections related to medical devices, such as intravascular catheters and indwelling prosthetic devices [1–3]. In addition, the emergence of antibiotic resistance in this microorganism has been documented. Infections with *S. epidermidis* cause a substantial prolongation of hospital stay and may eventually require removal of the indwelling foreign material [1].

*S. epidermidis* attaches to polymer surfaces by a complex process, and multiple factors may be involved at different stages of this process. Nearly all *S. epidermidis* strains can attach to polymer surfaces, although there are quantitative differences in attachment, depending on the characteristics of strains, the substratum of the surface, and the dynamic environment of the host [4]. After implantation, the polymer surface is rapidly covered with a conditioning film of various plasma and tissue fluid components (e.g., fibrinogen [Fg], fibronectin [Fn], vitronectin, and other proteins) [4]. Attachment of *S. epidermidis* is increased in the presence of matrix proteins (e.g., Fg and Fn), compared with the presence of bovine serum albumin, and is inhibited by prior proteolytic treatment of the bacteria, which suggests a functional involvement of surface proteins [5].

Infections caused by foreign bodies proceed in 2 steps [1, 6]: primary attachment, followed by cell accumulation on the polymer surfaces with biofilm formation. Several factors might contribute to the primary attachment of *S. epidermidis* to medical device-related polymer surfaces, including hydrophobic inter-

actions, capsular polysaccharide adhesion [7], autolysin [8], staphylococcal surface protein [9], and the cell wall-associated Fg binding protein, termed Fbe [10, 11]. It is believed that specific interactions between bacterial binding proteins and the conditioning film on the polymer surfaces overrule the non-specific forces [2]. The second step of bacterial attachment involves several factors, including polysaccharide intercellular adhesion and accumulation-associated protein [12]. It is believed that biofilm formation of the microorganisms plays an important role in this step and that the formation of biofilm may protect bacteria from the action of antibiotics and antibodies.

In previous studies, an *S. epidermidis* surface protein, termed Fbe, with affinity for Fg was identified. This protein can bind to both soluble and immobilized forms of Fg. The binding domain of Fg to Fbe is located on its  $\beta$ -chains. Addition of Fbe can competitively block bacterial adherence to immobilized Fg [11]. In this study, we have extended previous studies that address the biologic function of antibodies against Fbe, focusing on the direct interactions between antibodies and bacteria on in vitro- and in vivo-coated catheters.

### Materials and Methods

**Bacterial strains.** *S. epidermidis* 19, a clinical isolate from a patient with peritonitis, was studied because of its strong adherence to an Fg-coated surface.

**Generation of antibodies and their purification.** Rats were immunized with glutathione S-transferase (GST)-Fbe fusion protein or GST alone as a control. Purification of the antigens and immunization in rats has been described elsewhere [11]. Antibodies were purified by affinity to Protein G Sepharose 4 Fast Flow (Pharmacia), according to the manufacturer's protocol.

**Conditioning of catheters.** Polyethylene catheters with inside diameters of 2.92 mm and outside diameters of 3.73 mm (Becton Dickinson) were cut into 1-cm lengths and were coated with 0.2 mg/mL of Fg (FgCC) or Fn (FnCC) at room temperature overnight. Catheters of the same size were implanted subcutaneously in rats (SIC) for 24 h. This is the implantation time shown in pilot experiments to result in the highest bacterial adherence. Peripheral venous catheters (PVCs) were obtained from patients who had undergone intra-

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venous injections. These catheters were rinsed with PBS and were kept at  $-20^{\circ}\text{C}$  until used for bacterial adherence determination. Surface components bound to the surfaces were recovered from FgCC, SIC, and PVC by treatment with SDS-polyacrylamide gel electrophoresis loading buffer and were subjected to Western immunoblotting by probing with antibodies against Fg or Fn.

**Adherence assay.** Bacteria were grown in BHI medium at  $37^{\circ}\text{C}$  overnight to an OD at 600 nm of 1.0. A 1-mL bacterial suspension in PBS ( $5 \times 10^6$  cfu/mL) that had been pretreated with anti-GST-Fbe or anti-GST (control) at  $25 \mu\text{g/mL}$  was applied to the following conditioned catheters: FgCC, FnCC, and SIC. The incubations were done at room temperature for 2 h. After being rinsed with PBS, the catheters were sonicated in 1 mL of Luria broth for 4 min, which was a time that was found to be optimal. Adherent bacteria were determined by plating serial dilutions on blood agar plates. The PVC were cut into equal halves and were incubated as above with bacteria pretreated with anti-GST-Fbe or anti-GST, respectively. We used a paired Student's *t* test to compare the differences in blocking between the antibodies.  $P < .05$  was considered to be significant.

## Results

**Adherence conditions of *S. epidermidis* 19 to polyethylene catheters.** Bacteria adhered to the surface of catheters relatively rapidly, reaching 50% of the maximal levels within 15 min, and adherence was completed within 150 min. Bacteria also were shown to bind to the Fg-coated surfaces in a dose-dependent manner (data not shown).

**Fg was detected from different catheters.** The protein from an Fg-coated catheter was extracted, and 3 bands corresponding to the  $\alpha$ -,  $\beta$ -, and  $\gamma$ -chains of Fg were detected. Quantification, using standard amounts of Fg, led to an estimation of  $0.4 \mu\text{g}$ , corresponding to a coating density of  $\sim 67 \text{ ng/mm}^2$ . Extracted components from the SIC showed a number of proteins by silver staining; there were  $>30$  bands, with molecular sizes ranging from 6.5 to 175 kDa. In this sample, the  $\beta$ -chains of Fg, with an estimated quantity of  $0.1 \mu\text{g}$  (corresponding to a coating density of  $\sim 15 \text{ ng/mm}^2$ ), was detected by anti-Fg-horseradish peroxidase (anti-Fg-HRP). In the sample, which was extracted from PVC, the  $\beta$ -chain of Fg was the dominating component detected by anti-Fg-HRP from  $>30$  bands representing the adherent components on PVC (data not shown).

**Blocking of adherence with antibodies.** Antibodies raised against GST-Fbe were shown elsewhere [11] to inhibit the adherence of *S. epidermidis* to Fg-coated microtiter plates. Here, we determined the ability of antibodies against GST-Fbe to block adhesion of *S. epidermidis* to various types of catheters. We tested 3 types of catheters: some that had been coated with Fg in vitro (FgCC), some that had been implanted subcutaneously in rats (SIC), and some peripheral venous catheters obtained from human patients (PVC). Fg, especially the  $\beta$ -chain, was detectable from all of these catheters; however, there was a difference in density. In all cases, antibodies against GST-Fbe blocked adherence of *S. epidermidis*, but control antibodies

against GST did not. An 80% reduction in adherence was obtained for FgCC ( $P < .001$ ), 50% for SIC ( $P = .05$ ), and 60% for PVC ( $P < .01$ ; figure 1). The adherence-blocking effect is dose dependent: anti-GST-Fbe antibodies could be diluted 1000 times before losing their blocking ability (data not shown).

The adhesion of *S. epidermidis* to FgCC or FnCC was compared (figure 2). Anti-GST-Fbe showed inhibition of bacterial adherence only to the Fg-coated surfaces, whereas there was no such effect on FnCC. Pretreatment of bacteria with control antibodies had no blocking effect, compared with pretreatment with PBS (control B; figure 2).

## Discussion

Implanted biomaterials are covered by plasma components from host fluids, in which Fg appears to be abundant. Other studies also have confirmed that Fg was the only component consistently present on hemodialysis tubing from all patients, whereas Fn and von Willebrand factor were present irregularly, and vitronectin and thrombospondin remained undetectable [13]. On SIC from rats and on human PVC, Fg was detected among

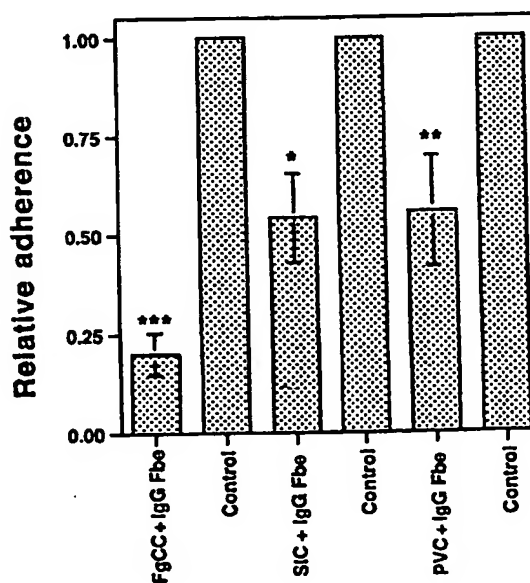


Figure 1. Blocking of adherence of *Staphylococcus epidermidis* to catheters by antibodies against a fibrinogen-binding protein, termed Fbe. Catheters were fibrinogen-coated in vitro (FgCC), subcutaneously implanted in rats (SIC), or peripheral venous catheters from human patients (PVC). Bacteria ( $2 \times 10^6$  cfu/mL) were pretreated with antibodies against glutathione S-transferase (GST)-Fbe or, as a control, with antibodies against GST before being applied to the conditioned catheters. Bacterial adherence was determined as described in Materials and Methods. Relative adherence was obtained by comparing adherent bacteria treated with anti-GST-Fbe to the control. Values shown are mean  $\pm$  SE of relative adherence from experiments done in triplicate. \* $P = .05$ ; \*\* $P < .01$ ; and \*\*\* $P < .001$ , using paired Student's *t* test.

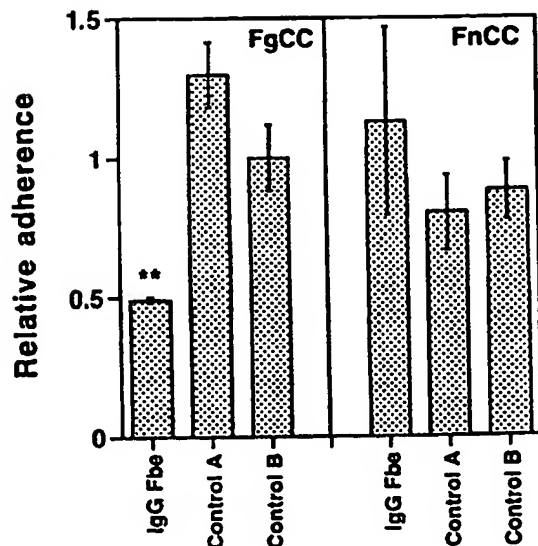


Figure 2. Antibodies against a fibrinogen-binding protein, termed Fbe, were specific in blocking *Staphylococcus epidermidis* adherence to fibrinogen (Fg)-coated but not to fibronectin (Fn)-coated surfaces. Bacteria ( $2 \times 10^4$  cfu/mL) pretreated with antibodies against glutathione S-transferase (GST)-Fbe or against GST (Control A) were added to catheters coated with Fg (FgCC) or Fn (FnCC). Bacteria not pretreated with antibodies also were applied to FgCC and FnCC (Control B). Relative adherence was obtained by comparing adherent bacteria treated with anti-GST-Fbe to control (bacteria bound to FgCC without antibodies). Results are the mean  $\pm$  SE of 3 catheter experiments; the experiment was done twice with similar results. \*\* $P < .01$ , compared with Control A.

other host components, but Fn was not found. Adherence of *S. epidermidis* to Fg-coated catheters is dose dependent. In our study, the amount of Fg recovered from implanted catheters was  $\sim 3$ -fold less than the amount recovered from in vitro coated catheters and resulted in  $\sim 3$ -fold less bacterial adhesion. Furthermore, adherence of *S. epidermidis* to Fg and to Fn seemed to be similar. Thus, Fg is a significant factor contributing to the adherence of *S. epidermidis* to catheters coated in vivo with plasma proteins. In our study, we mainly detected Fg  $\beta$ -chains from SIC and PVC, which is consistent with a previous finding that the  $\beta$ -chains of Fg are less sensitive to in vivo proteolytic degradation [14]. Fbe binds to the  $\beta$ -chains of Fg [11].

Most strains of *S. epidermidis* have a pronounced ability to bind nonspecifically to naked polymer surfaces. This binding can be blocked by coating the surface with various proteins, including Fg. However, binding of a strain, such as *S. epidermidis* 19, also is stimulated by Fg. Thus, Fg seems to have a dual effect: blocking of nonspecific binding and promotion of specific binding to Fg. Nonspecific binding is an important factor when implant materials become contaminated in vitro before implantation. Propagation and adherence of the progeny of infecting bacteria on polymer surfaces in vivo takes place at

a stage when the material has been implanted for some time and has become coated with plasma proteins.

Adherence of *S. epidermidis* to Fg was shown to be dependent on an Fg binding protein, Fbe [10, 11]. In this study we have tested the capacity of antibodies against Fbe to block adherence of *S. epidermidis* to Fg-CC, SIC, and PVC. In all cases, antibodies against GST-Fbe were shown to have a significant blocking effect, compared with that of control antibodies against GST (figure 1). The blocking effect was more pronounced ( $\sim 80\%$ ) on catheters coated solely with Fg than on catheters multiply coated in vivo with various plasma proteins (figure 1), of which all contribute to adherence. The anti-GST-Fbe antibodies were unable to block adherence to a surface coated with Fn (figure 2), which demonstrates a specificity in the blocking.

Taken together, these findings suggest that antibodies against Fbe might be used for prophylaxis against foreign body-associated infections caused by *S. epidermidis*. It is conceivable that antibodies against other structures on the surface of *S. epidermidis*, which mediates adherence to other plasma proteins, might contribute to further blocking.

In conclusion, we have demonstrated that adherence of *S. epidermidis* to FgCC, SIC, and PVC is dependent on the Fg adsorbed on the polymer surfaces and that this interaction can be blocked effectively by antibodies against Fbe.

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